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**(54) Food packaging film and methods of treating foodstuff**

Verpackungsfilm für Nahrungsmittel und Methoden zur Behandlung von Nahrungsmitteln

Film d'emballage et méthodes pour le traitement des aliments

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**Description**

[0001] The invention relates to antimicrobial and bactericidal composition containing packaging and processing films for foodstuffs containing such compositions and a method for inhibiting or preventing growth of microbes such as bacteria, molds, and yeasts on food surfaces.

[0002] "Food preservation", as that term is used herein, includes methods which guard against food poisoning as well as methods which delay or prevent food spoilage due to microbes. Food preservation keeps food safe for consumption and inhibits or prevents nutrient deterioration or organoleptic changes causing food to become less palatable.

[0003] "Food spoilage", as that term is used herein, includes any alteration in the condition of food which makes it less palatable including changes in taste, smell, texture or appearance. Spoiled food may or may not be toxic.

[0004] "Food poisoning", as that term is used herein, refers to mammalian disease caused by ingestion of food contaminated by pathogenic viruses, molds or bacteria and/or their toxins. Pathogen-contaminated food does not necessarily show any organoleptic sign of spoilage. Bacterial food poisoning may be caused by either infection of the host by the bacterial organism or by action of a toxin produced by the bacteria either in the food or in the host.

[0005] Prevention of food spoilage and food poisoning has been attempted throughout history often through trial and error. The early attempts have resulted in the adoption of such food preservation methods as the drying, salting and/or smoking of foods in order to preserve them. It has been relatively recent in recorded history that food preservation has been placed upon a scientific foundation. In the nineteenth century, work of such scientists as Louis Pasteur and Robert Koch elucidated the bacterial causes of food poisoning and spoilage and provided new methods of identifying pathogenic bacteria and of preserving food.

[0006] Present food technologists utilize an array of physical, chemical, and biological processes and agents to preserve food and prevent the transmission of disease via foodstuffs. In addition to such processes as irradiation, fermentation, pasteurization, control of temperature, pH and/or water activity, a plethora of chemical agents exist. These agents include antioxidants to prevent chemical degradation of food, as well as compositions which kill or inhibit deleterious bacteria and/or other microbes thereby preserving food i.e. preventing both spoilage and the transmission of disease. Commonly employed antimicrobial chemical agents include nitrates, nitrites, sulphur dioxide, sulfites, and acids such as acetic, propionic, lactic, benzoic, and sorbic acid and their salts, wood smoke and liquid smoke, and antibiotics such as natamycin and nisin.

[0007] Prevention of food poisoning is of paramount importance in the food processing industry. Concern for food safety has led most countries to heavily regulate the food industry to ensure public health. Also, manufacturers of processed food invest considerable resources to ensure the safety of their products. Despite these efforts, food poisoning still occurs. Many instances of food poisoning are attributed to bacteria such as *Salmonella*, *Clostridium*, and *Staphylococcus* among others.

[0008] Of rising concern is the relatively recent discovery in the food processing industry of widespread *Listeria* contamination of poultry and processed foods such as wieners, other sausages, cheese, dairy products including novelty ice cream, and seafood. Of particular concern is the recent evidence that pasteurized and fully cooked processed foods are being contaminated with microbes such as *Listeria monocytogenes* following cooking or pasteurization and prior to packaging for point of sale. Such contamination is typically surface contamination believed to be caused by contact of microbes with food surfaces subsequent to heat treatment (i.e. cooking or pasteurization). Microbes such as *Listeria* may be airborne (i.e. carried by dust) or present on food contact surfaces such as processing equipment.

[0009] In the 1980's several outbreaks of food poisoning have been reported worldwide in which the causative agent is suspected to be or has been identified as *Listeria* contaminated food. Outbreaks of listeriosis (infection by *Listeria* bacteria) in humans have been reported in Massachusetts, California, and Pennsylvania in the U.S.A. and also in Canada, and Switzerland. These outbreaks have been attributed to ingestion of *Listeria* contaminated food such as coleslaw, cheese made from raw milk, surface ripened soft cheeses, and salami. Hundreds of people have been affected with a mortality rate of up to about one third of those affected. Particularly susceptible to the disease (which is contagious) are pregnant women, fetuses, newborn and infant children as well as adults with compromised immune systems e.g. adults under treatment with immunosuppressive drugs such as corticosteroids. Listeriosis is a serious disease which may cause meningitis, spontaneous abortion, and perinatal septicemia. Although treatable with early diagnosis, untreated listeriosis exhibits a high mortality rate.

[0010] Food preservation by inhibition of growth of *Listeria monocytogenes* is difficult. *Listeria* can reportedly reproduce and grow both aerobically and anaerobically, at pHs above 4.85 and over a wide range of temperatures which can be as low as 3°C and as high as about 45°C. This means that *Listeria* can grow at normal refrigeration temperatures. *Listeria* has also been reported as being able to grow in a water solution of up to 10% salt. Fortunately, cooking or pasteurization kills *Listeria*. Unfortunately, microorganism contamination may occur following pasteurization by the processor. Many people eat processed foods after a significant period of time has elapsed from a first cooking or pasteurization by the food manufacturer thereby permitting bacteria introduced by post-pasteurization contamination to grow. Since this food consumption may occur without reheating the processed food to sufficient temperatures for

sufficient time to kill any microbes (such as Listeria) which may have been introduced subsequent to initial cooking, there is a risk of food poisoning. The present invention seeks to ameliorate the aforementioned risk.

[0011] WO-A-89/12399 is a novelty-only document according to Article 54 (3) EPC for all contracting states except Spain, but only to the extent of the first priority document of WO-A-89/12399 (i. e. US 209 861). WO-A-89/12399 and US 209 861, respectively, describe a bacteriocidal composition of nisin and a chelating agent and its use as a food preservative by dipping the food to be treated into a solution of such a combination. Said reference is void of the idea of transferring the bactericidal composition to the food surface to be treated via food packaging films.

[0012] It is an object of the invention to provide food packaging or processing films containing a bactericidal composition comprising in combination a Streptococcus lactic-derived or synthetic equivalent bacteriocin such as nisin, and a chelating or sequestering agent.

[0013] It is an object of the invention is to provide a film containing a heat-resistant antimicrobial composition comprising a Streptococcus lactic-derived or synthetic equivalent bacteriocin such as nisin and a chelating or sequestering agent.

[0014] Another object of the invention is to provide a film containing an antibacterial composition effective to kill and/or inhibit growth of Listeria.

[0015] It is an object of the invention to provide a polymeric film capable of transferring a controlled amount of an antimicrobial composition to a foodstuff surface.

[0016] Another object of the invention is to kill, inhibit or prevent the growth of pathogenic microorganisms on the surface of a foodstuff by a method of transferring an antimicrobial composition to a surface of a foodstuff in a controlled amount.

[0017] Another object of the invention is to transfer an antimicrobial composition to a foodstuff in an amount effective to prevent growth of pathogenic bacteria on the surface of the foodstuff after removal of the film for the normal shelf life of the foodstuff.

[0018] Another object of the invention is to prevent or inhibit growth of Listeria monocytogenes on skinless wieners following initial cooking and casing removal, through packaging for consumer sale until opening of the packaged wieners for consumption by the consumer.

[0019] Another object of the invention is to increase shelf life of processed foods by application of a synergistic mixture, preferably a liquid or a suspension, of a Streptococcus lactic-derived (or synthetic equivalent) bacteriocin and a chelating agent.

[0020] The foregoing objects will be achieved by the subject-matter of claims 1 to 16.

[0021] The foregoing objects and others which will become apparent from that which follows may be achieved by treating a foodstuff, preferably a foodstuff surface, with an antimicrobial composition comprising a chelating agent such as EOTA in synergistic combination with a Streptococcus-derived bacteriocin such as nisin, the treatment being performed by contacting the foodstuff with a film containing said antimicrobial agent. The film may hold the composition in contact with the foodstuff surface thereby transferring a controlled amount of the composition from the film to the surface of the foodstuff or it may transfer the antimicrobial composition (with or without use of a transfer agent such as zein) to the foodstuff whereby the film may be removed with transferred antimicrobial composition remaining on the foodstuff surface in an effective amount to kill, prevent or inhibit growth of either food spoilage organisms or pathogenic microorganisms such as Listeria thereon.

[0022] In one aspect of the invention a food packaging film is provided which comprises a polymeric film containing a bactericidal composition comprising a heat-resistant, Streptococcus-derived bacteriocin (or synthetic equivalent) antibacterial agent. This agent is preferably effective against growth of gram positive bacteria, especially Listeria monocytogenes, and may be a heat resistant antibiotic or bacteriocin such as nisin or bacteriocin PA-1. Preferably, the heat resistant agent is nisin.

[0023] It is not necessary that each and every one of the above objects be present in all embodiments of the invention; it is sufficient that the invention may be advantageously employed.

[0024] Fundamental to the composition of the invention is the synergistic combination of a sequestering or chelating agent such as an EDTA salt or citric acid with a Streptococcus-derived bacteriocin (or synthetic equivalent) such as nisin. A liquid mixture or suspension of nisin and chelating agent is especially preferred.

[0025] A fundamental aspect of one method of the present invention is the protection of the foodstuff surface for a substantial period of time after removal of the film.

[0026] A fundamental aspect of one embodiment of the inventive film is the use of a heat resistant antimicrobial agent which is effective against bacteria after pasteurization or heat treatment.

[0027] An antimicrobial composition comprising a synergistic combination of a Streptococcus-derived bacteriocin such as nisin (or a synthetic equivalent) and a chelating agent such as citric acid has been discovered to have unexpectedly good bactericidal properties especially against pathogenic bacteria such as Listeria monocytogenes. Additionally, the composition surprisingly is able to prolong food shelf life by preventing food spoilage for a longer period of time than would be expected based upon the effectiveness of either component alone.

[0028] Nisin is the preferred bacteriocin. Nisin is a polypeptide bacteriocin produced by the lactic acid bacteria, *Streptococcus lactis* Group N.

[0029] Nisin is reportedly a collective name representing several closely related substances which have been designated A, B, C, D and E nisins having a similar amino acid composition. The structure and properties of nisin are further discussed in the article by E. Lipinska, entitled "Nisin and Its Applications", *The 25th Proceedings of the Easter School in Agriculture Science at the University of Nottingham, 1976*, pp. 103-130 (1977), which article is hereby incorporated by reference. The World Health Organization Committee on Biological Standardization has established an international reference preparation of nisin, and the international unit (IU hereinafter) is defined as 0.001 mg of this preparation. NISAPLIN is the brand name for a Nisin concentrate containing 1 million IU per gram which is commercially available from Aplin & Barrett Ltd., Trowbridge, Wiltshire, England.

[0030] Nisin is a known food preservative which is also known to be heat-stable, acid-stable and active against gram-positive bacteria. Nisin is used as a food preservative in dairy products and vegetables usually in conjunction with heat treatment. Nisin also occurs naturally in raw milk and has been used in heat processing of meat pastes. Nisin is considered to be nontoxic with toxicological data indicating no adverse effect at levels of 3.3 million IU per Kg of body weight. Nisin reportedly can stand heating to 121°C without loss of activity. Although some loss of activity may be expected when used with processed foods, this may be ameliorated e.g. by increasing the amount of nisin applied. Effective levels of nisin to preserve foodstuffs reportedly range from 25-500 IU/g or more.

[0031] Suitable chelating (sequestering) agents include carboxylic acids, polycarboxylic acids, amino acids and phosphates. In particular, the following compounds and their salts are among those believed to be useful:

20 Acetic acid

Adenine

Adipic acid

ADP

25 Alanine

B-Alanine

Albumin

Arginine

Ascorbic acid

30 Asparagine

Aspartic acid

ATP

Benzoic acid

35 n-Butyric acid

Casein

Citraconic acid

Citric acid

Cysteine

40 Dehydracetic acid

Desferri-ferrichrysin

Desferri-ferrichrome

Desferri-ferrioxamin E

45 3,4-Dihydroxybenzoic acid

Diethylenetriaminepentaacetic acid (DTPA)

Dimethylglyoxime

O,O-Dimethylpururogallin

50 EDTA

Formic acid

Fumaric acid

Globulin

55 Gluconic acid

Glutamic acid

Glutaric acid

Glycine

	Glycolic acid Glycylglycine Glycylsarcosine Guanosine
5	Histamine Histidine 3-Hydroxyflavone Inosine
10	Inosine triphosphate Iron-free ferrichrome Isovaleric acid Itaconic acid
15	Kojic acid
	Lactic acid Leucine Lysine
20	Maleic acid Malic acid Methionine Methylsalicylate
25	Nitrilotriacetic acid (NTA)
	Ornithine Orthophosphate Oxalic acid Oxystearin
30	B-Phenylalanine Phosphoric acid Phytate
35	Pinelic acid Pivalic acid Polyphosphate Proline
40	Propionic acid Purine Pyrophosphate Pyruvic acid Riboflavin
45	Salicylaldehyde Salicyclic acid Sarcosine Serine
50	Sorbitol Succinic acid
	Tartaric acid Tetrametaphosphate
55	Thiosulfate Threonine Trimetaphosphate Triphosphate

Tryptophan

Uridine diphosphate  
Uridine triphosphate

5

n-Valeric acid  
Valine

Xanthosine

10 [0032] Many of the above sequestering agents are useful in food processing in their salt forms which are commonly alkali metal or alkaline earth salts such as sodium, potassium or calcium or quaternary ammonium salts. Sequestering compounds with multiple valencies may be beneficially utilized to adjust pH or selectively introduce or abstract metal ions e.g. in a food system coating. Additional information on sequestering and chelating agents is disclosed in T. E. Furia (Ed.), CRC Handbook of Food Additives, 2nd Ed., pp. 271-294 (1972, Chemical Rubber Co.), and M.S. Peterson and A.M. Johnson (Eds.), Encyclopedia of Food Science, pp. 694-699 (1978, AVI Publishing Company, Inc.) which articles are both hereby incorporated by reference.

15 [0033] The terms "chelating agent" and "sequestering agent" are used herein as synonyms and are defined as organic or inorganic compounds capable of forming coordination complexes with metals.

20 [0034] Also, as the term "chelating agent" is used herein, it includes molecular encapsulating compounds such as cyclodextrin. The chelating agent may be inorganic or organic, but preferably is organic.

[0035] Preferred chelating agents are nontoxic to mammals and include aminopolycarboxylic acids and their salts such as ethylenediaminetetraacetic acid (EDTA) or its salts (particularly its di- and tri-sodium salts), and hydrocarboxylic acids and their salts such as citric acid. However, non-citric acid and non-citrate hydrocarboxylic acid chelating agents are also believed useful in the present invention such as acetic acid, formic acid, lactic acid, tartaric acid and and their salts.

25 [0036] As noted above, the term "chelating agent" is defined and used herein as a synonym for sequestering agent and is also defined as including molecular encapsulating compounds such as cyclodextrin. Cyclodextrins are cyclic carbohydrate molecules having six, seven, or eight glucose monomers arranged in a donut shaped ring, which are denoted alpha, beta or gamma cyclodextrin, respectively. As used herein, cyclodextrin refer to both unmodified and modified cyclodextrin monomers and polymers. Cyclodextrin molecular encapsulators are commercially available from American Maize-Products of Hammond, Indiana. Cyclodextrin are further described in Chapter 11 entitled, "Industrial Applications of Cyclodextrin", by J. Szefli, page 331-390 of Inclusion Compounds, Vol. III (Academic Press, 1984) which chapter is hereby incorporated by reference.

30 [0037] Mixtures of Streptococcus-derived bacteriocins, such as nisin, with one or more chelating agents may be usefully employed according to the present invention. Such mixtures may be solid in liquid suspensions or solutions. Unless otherwise noted, use of the term "solution" herein includes not only solids or liquids dissolved in a liquid but also solid-in-liquid suspensions or mixtures. Suitable solvents, diluents or carriers for the mixture of chelating agent and bacteriocin are water, alcohols, propylene glycol, oils such as mineral, animal or vegetable oil, glycerine or lecithin.

35 [0038] Although the commercially available bacteriocins may contain dairy products it may be advantageous for the bactericidal composition or food preservation preparation of the present invention to contain no added dairy products such as cheese, whey, curds or calcium-containing milk-based solids. It has been reported that calcium and magnesium ions may inactivate nisin. It may be, without wishing to be bound by the belief, that agents which chelate calcium and/or magnesium may be particularly advantageous. Mixtures containing a mixture of bacteriocin and chelating agent may be applied to foodstuffs including dairy products and nondairy products such as sausages, other meats, vegetables and fruits by use of an impregnated or coated film as described below. Such solutions may be formulated with widely varying pHs, but advantageously will be neutral or acidic. Acidic solutions are believed to enhance or maintain the antibacterial effective of these novel solutions and are therefore preferred. Solutions having a pH less than or equal to about 6 are preferred, and less than or equal to 5 especially preferred. Amounts of the bacteriocin and chelating agent components may vary depending upon such factors as: type of bacteriocin type of chelating agent, pH, other constituents present (e.g. type of solution solvent), application i.e. type of foodstuff to which the materials are being applied, how applied (e.g. mixing with foodstuff or surface coating, subsequent processing conditions (e.g. heat treatment), a desired time period of effectiveness to kill or inhibit bacteria, and type of bacteria the foodstuff is being protected against, etc. One of ordinary skill in the art may determine appropriate amounts of bacteriocin and chelating agent without undue experimentation. Water is the preferred solvent for preparing a solution. Suitable amounts of bacteriocin in a mixture for treating foodstuffs such as sausage include from 5 to 250 ppm bacteriocin (by weight of total mixture) or more. Amounts less than 5 ppm are workable, but depending upon use may be less effective than higher concentration. Amounts greater than 250 ppm are also workable, but increasing concentrations have the disadvantage of increasing costs due to the expense of the bacteriocin. Concentrations between 50 and 150 ppm have been found to

be effective and inexpensive, with concentrations of 150 ppm or more very effective at killing or inhibiting such pathogenic bacteria as *Listeria monocytogenes* e.g. on cooked frankfurter surfaces. The inventive solution may be used against other bacteria and is especially effective against gram positive bacteria. The amounts of chelating agent used may vary widely e.g. amounts between about 0.2 to about 0.8 or 3.0 weight percent or more may be usefully employed.

5 The composition may also contain other antimicrobial or antibacterial agents, or other additives such as colorants and flavorants e.g. gaseous or liquid smoke.

[0039] Food packaging films suitable for use in the present invention include polymeric films such as blown film, oriented film, stretch and shrink film, heat shrinkable bags and food casings. "Food packaging films" as that term is used herein are flexible sheet materials which are suitably 15 mils or less and preferably less than 10 mils (25 microns) 10 in thickness.

[0040] Suitable films include regenerated cellulose and thermoplastic stretch or shrink films, and may be monolayer or multilayer films. Shrink films are preferably formed into heat shrinkable, biaxially-oriented bags.

[0041] Suitable films include food casings which are generally flexible films which are preferably tubular and may be formed of polymeric materials including cellulosic materials such as regenerated cellulose or cellulose carbamate or of plastics such as homopolymers or copolymers of polyolefins e.g. polypropylene, polyethylene, or polyamides, polyethylene terephthalate, polyvinylidene chloride copolymers or ethylene-vinyl acetate copolymers or of proteinaceous films such as collagen. Preferably, casings are tubular cellulosic casings, that are prepared by any one of the methods well known in the art. Such casings are generally flexible, thin-walled seamless tubing which is preferably formed of regenerated cellulose, and the like, in a variety of diameters. Also suitable are tubular cellulosic casings having a fibrous reinforcing web embedded in the wall thereof. Casings having a reinforcing web are commonly called "fibrous food casings", whereas cellulosic casings without the fibrous reinforcement, are herein referred to as "non-fibrous" cellulosic casings. Both natural and synthetic casings are contemplated by the present invention.

[0042] Casings conventionally known as "dry stock casings" may be used in the practice of this invention. Such casings generally have a water content within the range of from about 5 to about 14 weight percent water if non-fibrous 25 casing, or within the range of from about 3 to about 8 weight percent water if fibrous casing, based on the total weight of casing including water.

[0043] Casings conventionally known as "gel stock casings" are casings which have higher moisture contents since they have not been previously dried, and such casings may also be used in the practice of this invention.

[0044] Casings conventionally known as "remoisturized casings" are dry stock casings to which moisture has been 30 added, for example, to facilitate stirring and/or stuffing, and such casing may be used in the practice of this invention. Such casings generally have a water content within the range of about 15 to about 23 weight percent if non-fibrous casing, or within about 16 to about 35 weight percent if fibrous casing, based on the total weight of the casing including the water.

[0045] Antimicrobial agents suitable for use in the invention include any agents which may be effectively transferred 35 from a food packaging film to a foodstuff to provide a foodstuff surface containing an antimicrobial agent which prevents or inhibits growth of microorganisms thereon, preferably even after removal of the film from contact with the foodstuff surface.

[0046] Suitable antimicrobial agents may be effective against molds, yeasts and/or bacteria. Suitable agents are believed to include antibacterial agents, which are effective to kill or inhibit bacteria, such as antibiotics e.g. nisin, 40 natamycin, subtilin, or *Streptococcus*-derived bacteriocin, heat resistant enzymes such as lysozyme and other agents such as liquid smoke, parabens, sorbic acid, benzoic acid and mixtures thereof.

[0047] Such antimicrobial agents may include additives such as binding agents, buffers, emulsifiers, transfer aids or chelating agents such as ethylenediaminetetraacetic acid (EDTA) or its salts. These agents may enhance the antimicrobial effect of the agent or assist transfer from the packaging film to the foodstuff.

[0048] In particular, binding agents, e.g. water insoluble agents such as shellac and zein, may be used as transfer 45 means or agents to provide transfer of an antimicrobial agent incorporated therewith from a packaging film to a foodstuff surface under moisturizing conditions. Preferred binding or transfer agents will have preferential substantivity to the foodstuff surface relative to the packaging film.

[0049] The food packaging film of the present invention will contain on or within the film an antimicrobial, preferably 50 antibacterial, agent. Advantageously, such agent will be effective to kill, inhibit or prevent the growth of bacteria such as those from the genera *Listeria*, *Salmonella* and *Clostridium* and preferably the species *Listeria monocytogenes*.

[0050] Preferred antibacterial agents will be resistant to destruction or inactivation by heat treatment such as cooking or pasteurization temperatures and times. It is necessary that such preferred agents be able to survive heat treatment of the foodstuff within a packaging film and be effective subsequent to the heat treatment and removal of the film.

[0051] "Heat resistant", as that term is used herein, means that the antimicrobial agent, which is capable of being applied to a film in a controlled amount, withstands destruction, inactivation or losses due to heat treatment, e.g. by pasteurization or cooking, such that following heat treatment sufficient agent remains which is effective to kill, inhibit or prevent growth of microorganisms on foodstuffs to which it is applied. It should be understood that partial losses in

the amount of agent or agent effectiveness may occur and that partial inactivation may also occur. However, it is sufficient that the remaining active agent be capable of protecting the foodstuff surface against pathogenic organisms such as Listeria.

[0052] In a typical application of the invention a tubular cellulosic food casing which may be impregnated or coated with an antimicrobial agent is used in the production of skinless wieners. In this well known sausage pasteurization process, the casing is stuffed with meat emulsion and formed into links. The encased meat emulsion which is given its shape by the casing is then cooked (heat treated) at a suitable temperature for sufficient time to achieve pasteurization. Typically, meat products, including sausages, are kept (even during pasteurization) at temperatures below about 190°F (88°C) prior to sale for home or institutional use. Generally, during pasteurization foodstuffs, such as processed meat, will be brought to an internal temperature above about 145°F (63°C) and no higher than about 180°F (82°C) before removal of any film used to shape the product during processing. The surface temperature of these pasteurized foodstuffs typically does not exceed 190°F (88°C) and usually below 170°F (77°C). The encased wieners may then be further processed or treated e.g. by showering with water and/or chilling. The casing is then removed by well known peeling means from the heat treated wieners and the wieners are repackaged for retail sale.

[0053] In one embodiment of the invention, the casing is a peelable casing which is particularly adapted for high speed peeling and removal by machinery. Casing is known in the art to be peelable by hand or by machines. Not all casing which is peelable by hand is adapted for the high speed peeling performed by machines such as the well known Apollo Peeler manufactured by the Ranger Tool Company, Inc. of Bartlett, Tennessee. This and similar commercial peelers are able to remove casing from 40,000 to 60,000 sausages per hour.

[0054] Thus, the antimicrobial agent which is applied to the casing prior to stuffing must withstand the effects of this heat treatment and processing steps and remain effective to kill, inhibit, or prevent the growth of undesirable microorganisms following the heat treatment. Also, the agent must transfer to the foodstuff in an effective amount because the casing is removed. Microbial contamination of the wiener surfaces may occur in the time after removal of the casing and prior to packaging.

[0055] Alternatively, an antimicrobial agent such as the inventive antibacterial composition may be applied to a foodstuff via a coating on a film which is applied following heat treatment.

[0056] Therefore, the transferred or applied agent should be present in a sufficient amount and remain sufficiently effective following heat treatment, processing and casing removal to kill, inhibit or prevent growth of microbes, preferably *Listeria monocytogenes* for a sufficient length of time. This length of time for effectiveness for film applied agents should extend at least from the time of casing removal to packaging for sale e.g. to consumers or institutions. Advantageously, the agent would remain effective through the normal "sell by" or "expiration" date during which the food product is offered for sale by the retailer. Preferably, the effective time would extend past opening of the package by the consumer until the end of normal freshness period when food spoilage becomes apparent. For skinless wieners typical times are: about ten minutes to one hour from casing removal to consumer packaging, about thirty days to sixty days from consumer packaging through normal retail sale, and about seven days or more from opening of consumer package under normal refrigerated storage and use. In any case, the desired lengths of time and normal shelf life will vary from foodstuff to foodstuff and those of ordinary skill in the art will recognize that packaging times and shelf-life times will vary depending upon the type of foodstuff (e.g. beef wieners, poultry, or cheese), the size of the foodstuff, the number of pieces packaged (consumer size or institutional size package) storage temperatures, processing conditions and packaging equipment.

[0057] Transfer of an antimicrobial agent from the inner surface of a film in direct contact with a contiguous foodstuff surface, according to one embodiment of the invention, is such that the agent is at least in part permanently transferred to the foodstuff during processing thereof in an amount sufficient to be effective to kill or inhibit growth of *Listeria* bacteria on the foodstuff surface notwithstanding subsequent peeling and removal of the casing.

[0058] The above example is exemplary and should not be taken as limiting the invention to use with wieners. The invention is applicable to any foodstuff particularly those which may benefit from application of a controlled amount of an antimicrobial agent to the foodstuff surface, particularly an antibacterial agent. It is contemplated that the compositions, films and methods of the invention have applicability to both animal-derived and plant-derived foodstuffs including but not limited to sausages of all types (such as beef, pork, chicken, turkey, fish, etc.) primal and subprimal cuts of meat, luncheon meats, hams, lamb, steak, hamburger, and poultry including chicken, turkey, duck, goose, as well as fish, and dairy products such as semi-soft and hard cheeses, processed cheese, and vegetable products including lettuce, tofu, coleslaw, soybean derived protein substitutes for meat, etc.

[0059] Advantageously, the film and/or method of the present invention may utilize an antimicrobial, preferably antibacterial, heat resistant agent such as nisin. Nisin is a polypeptide bactericidic as described above.

[0060] The antimicrobial agents utilized according to the method and film of the present invention may be applied prior to or after film formation to intersperse, coat and/or impregnate the film with a controlled amount of agent per unit area of film. Mixtures of agents may also be used or a plurality of agents may be applied serially. Chelating agents, binding agents, emulsifiers and other additives may similarly be applied to the casing simultaneously (either in a mixture

or separately), or may be applied serially.

[0061] In the present invention the antimicrobial agent and additives may be applied to the outer surface of a film such as a tubular casing by passing the casing through a bath of a solution containing the agent and/or additives. The agent may be allowed to soak into the casing prior to doctoring off any excess liquid by passing the casing through squeeze rolls or wipers, and the like, for an amount of time sufficient for the casing to incorporate the desired amount of agent and additives. The process of passing the casing through a treatment bath, (which may also be referred to as a "dip bath" or a "dip tank,") may also be referred to as a "dipping" step. The agent additives may alternatively be externally applied to the casing by methods other than dipping, such as spraying, brushing, roll-coating, printing and the like.

10 [0062] Alternatively, the agent or additives may be applied to the internal surface of a film tube such as a casing by any of several well-known procedures described in U.S. Pat. No. 4,171,381 to Chiu, the disclosure of which is incorporated by reference. These include slugging or bubble coating, spraying, and coating while stirring.

[0063] The slugging method for coating the inside of a casing involves filling a portion of the casing with the coating material, so that the slug of coating material generally resides at the bottom of a "U" shape formed by the casing being draped over two parallel rollers, and then moving the continuous indefinite length of casing so that the slug of coating material remains confined within the casing, while the casing moves past the slug and is coated on its inside wall by the coating material contained within the slug.

[0064] Also, there is disclosed, for example, in U.S. Pat. No. 3,451,827 a spraying method for applying a variety of coating materials over the internal surface of small diameter casings. In U.S. Pat. No. 3,378,379 to Shiner et al, a "slugging" method is used for applying coating materials to the internal surface of large diameter casings.

[0065] The antimicrobial agent may be applied to either side of the film as long as the film surface adapted for contact with the foodstuff is capable of allowing transfer of the agent to the foodstuff. For example, a cellulosic tubular food casing may be coated on the inside by slugging with a solution containing the agent as a solute or dispersion, or by spraying a controlled amount in either dry or liquid form. Then the inside of the casing may be stuffed with a food product such as hams, meat emulsion, or cheese in order to contact the foodstuff with the agent. Alternatively, the outer surface of the casing may be coated with the agent and the casing may be reverse stuffed by means well known (see e.g. U.S. Patent 4,162,693) in the art to contact the surface of the foodstuff with the agent.

[0066] It is to be noted that the agent which is coated on the film surface, whether externally coated or internally coated, may or may not exist solely as a surface coating. For example, the agent may penetrate the cellulosic structure of a casing as the cellulose absorbs a liquid solvent of the solution containing the agent. Alternatively, a nonabsorptive thermoplastic film, or a cellulosic film having a barrier coating which prevents impregnation or a multilayer film which allows partial impregnation up to a barrier layer may be utilized. Accordingly, as used herein, the term "coating" is to be understood to mean that the film wall is not necessarily impregnated but may only have the antimicrobial agent on the surface thereof, but the term may also apply where the film wall is interspersed or impregnated with the agent. In any case, in use the agent should be releasable from the film and transferable to a foodstuff surface to the extent necessary to provide an antimicrobial effect on the surface of the foodstuff.

[0067] Solutions which contain antimicrobial agents may, according to the present invention, also contain other ingredients which may be suitably used in treating a film. For example, a tubular food casing, may be coated with e.g., glycerine and/or propylene glycol which may function as humectants or softening agents, and the like, either in a 40 solution with the antimicrobial agent or separately.

[0068] Other ingredients which are normally used in the manufacture of, or for further treatment of, the food packaging film may also be present in or on the film if desired, and they may be used in the same manner and amounts as if the antimicrobial agent had not been used. For example, cellulose ethers and mineral oil are frequently used with cellulosic casings and antiblock and anti-static agents are frequently used with thermoplastic films.

45 [0069] The film may be in sheet form or tubular. It may be in rolls or cut lengths. Tubular film may be formed into bags by conventional techniques e.g. heat sealing and cutting across a tube or may be shirred. Film may be shirred by conventional methods.

[0070] Certain types of films such as regenerated cellulosic casing prior to shirring, may be dried and/or humidified to a water content suitable for shirring and/or further processing. The need for conventional drying and/or humidification depends on the water content of the casing after treatment, the type of casing, and the end use. Gel stock, dry stock and remoisturized casings may all be suitably employed in the present invention.

50 [0071] The invention will now be more clearly understood by reference to the following examples which are set forth as being merely illustrative of the invention and which are not intended, in any manner, to be limitative thereof. Unless otherwise indicated, all parts and percentages are by weight and all film or casing related percentages are based on the total weight of the film or casing. Bacterial plate counts are an arithmetic average for three plates unless otherwise indicated. Estimated plate counts were made by generally accepted procedures in the microbiological art.

Examples 1-16

[0072] The following examples 1-16 describe bactericidal compositions which can be used for the inventive films and methods.

- 5 [0073] The effectiveness of various test solutions and concentrations of the antimicrobial agents nisin were examined by a liquid assay method against growth of pathogenic bacteria such as the gram-positive bacteria Listeria monocytogenes. Growth of total aerobic bacteria was also measured and the effectiveness similarly examined. Use of the chelating agents ethylenediaminetetraacetic acid (disodium salt), citric acid, and cyclodextrin were also examined alone and with various concentrations of nisin.
- 10 [0074] These examples were conducted using aseptic techniques well known to those skilled in the art of microbiology. Sterilized DIFCO brand tryptose broth at double strength was inoculated with at least about 10,000 colony forming units (cfu) per ml. of a mixture of two pathogenic strains of food isolated Listeria monocytogenes serotype 4b. This inoculated broth was then added to test tubes containing double strength antimicrobial test solutions using equal portions of inoculated broth and test solution. The test tubes were then capped and the contents thoroughly mixed.
- 15 Tables 1a and 1b list test solution components and amounts. Following the test, the pH was measured for similarly mixed solutions of uninoculated broth and test solutions with the pH values also reported in Tables 1a and 1b. The amounts for the test solution components were calculated based on a "double strength" solution which was then diluted with an equal volume of inoculated broth as described above. The amounts listed in Tables 1a and 1b were calculated assuming equal weights for equal volumes of the inoculated broth and the test solutions. The mixed inoculated test solutions were made in triplicate and incubated without agitation at about 30°C. For each test sample, an aliquot of 0.3 ml was aseptically withdrawn by pipet directly following mixing (zero (0) hours) as well as at 4, 8, 24 and 48 hours. Directly following removal, these aliquots were plated out on LPM agar and tryptic soy agar plates according to standard plate count procedures known to those skilled in the art of microbiology to determine Listeria and total aerobic bacterial counts. The selective listeria count was made using the U.S. Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS), Microbiology Division method entitled, "FSIS Method for the Isolation and Identification of Listeria Monocytogenes From Processed Meat and Poultry Products" (For Use in Interim Laboratory Recognition Program) as described in the above titled paper, dated November 4, 1988 by A. B. Moran and dated November 8, 1988 by R. W. Johnston of FSIS and available from the FSIS which is hereby incorporated by reference. The bacterial count results for particular test solution components and concentrations are reported in Tables 1a and 1b as an arithmetic average bacterial count of colony forming units (cfu) per ml for three replicate plates.
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Table Ia

Example No.	Test Solution Components		Amount of B (wt.%)	Time (hr)	pH Listeria count (cfu/ml)			
	A (wt.%)	B (wt.%)			0	4	8	24
1	water	100	--	--	7.2 <10	10,000,000 <10	300,000,000 <10	7,700,000 <10
2	water	100	--	--	7.3 31,000	37,000 10,000,000	69,000 300,000,000	7,700,000 11,000
3	--	--	Na <sub>2</sub> EDTA 0.8	6.6 20,000	11,000 2,000	35,000 180	730 <10	730 <10
4	--	--	citric acid 0.8	4.2 11,000	<10 <10	<10 <10	<10 <10	<10 <10
5	nisin	0.0001	--	--	7.2 <10	<10 <10	<10 <10	<10 <10
6	nisin	0.0010	--	--	7.3 <10	<10 <10	<10 <10	<10 <10
7	nisin	0.0050	--	--	7.2 <10	<10 <10	<10 <10	<10 <10
8	nisin	0.0125	--	--	7.1 <10	<10 <10	<10 <10	<10 <10
9	nisin	0.0001 Na <sub>2</sub> EDTA	0.8 6.5	6.5 <10	<10 <10	<10 <10	<10 <10	<10 <10
10	nisin	0.0010 Na <sub>2</sub> EDTA	0.8 6.7	6.7 <10	<10 <10	<10 <10	<10 <10	<10 <10
11	nisin	0.0050 Na <sub>2</sub> EDTA	0.8 6.7	6.7 <10	<10 <10	<10 <10	<10 <10	<10 <10
12	nisin	0.0125 Na <sub>2</sub> EDTA	0.8 6.7	6.7 <10	<10 <10	<10 <10	<10 <10	<10 <10
13	nisin	0.0001 citric acid 0.8	4.4 <10	<10 <10	<10 <10	<10 <10	<10 <10	<10 <10
14	nisin	0.0010 citric acid 0.8	4.2 <10	<10 <10	<10 <10	<10 <10	<10 <10	<10 <10
15	nisin	0.0050 citric acid 0.8	4.6 <10	<10 <10	<10 <10	<10 <10	<10 <10	<10 <10
16	nisin	0.0125 citric acid 0.8	4.3 10	<10 <10	<10 <10	<10 <10	<10 <10	<10 <10

Example No.	Test	Solution Components	pH	Total Aerobic Bacteria Count (cfu/ml)
	A	Amount	Time (hr)	
1a	1a	1a	1a	1a
1b	1b	1b	1b	1b
1c	1c	1c	1c	1c
1d	1d	1d	1d	1d
1e	1e	1e	1e	1e
1f	1f	1f	1f	1f
1g	1g	1g	1g	1g
1h	1h	1h	1h	1h
1i	1i	1i	1i	1i
1j	1j	1j	1j	1j
1k	1k	1k	1k	1k
1l	1l	1l	1l	1l
1m	1m	1m	1m	1m
1n	1n	1n	1n	1n
1o	1o	1o	1o	1o
1p	1p	1p	1p	1p
1q	1q	1q	1q	1q
1r	1r	1r	1r	1r
1s	1s	1s	1s	1s
1t	1t	1t	1t	1t
1u	1u	1u	1u	1u
1v	1v	1v	1v	1v
1w	1w	1w	1w	1w
1x	1x	1x	1x	1x
1y	1y	1y	1y	1y
1z	1z	1z	1z	1z
1aa	1aa	1aa	1aa	1aa
1bb	1bb	1bb	1bb	1bb
1cc	1cc	1cc	1cc	1cc
1dd	1dd	1dd	1dd	1dd
1ee	1ee	1ee	1ee	1ee
1ff	1ff	1ff	1ff	1ff
1gg	1gg	1gg	1gg	1gg
1hh	1hh	1hh	1hh	1hh
1ii	1ii	1ii	1ii	1ii
1jj	1jj	1jj	1jj	1jj
1kk	1kk	1kk	1kk	1kk
1ll	1ll	1ll	1ll	1ll
1mm	1mm	1mm	1mm	1mm
1nn	1nn	1nn	1nn	1nn
1oo	1oo	1oo	1oo	1oo
1pp	1pp	1pp	1pp	1pp
1qq	1qq	1qq	1qq	1qq
1rr	1rr	1rr	1rr	1rr
1ss	1ss	1ss	1ss	1ss
1tt	1tt	1tt	1tt	1tt
1uu	1uu	1uu	1uu	1uu
1vv	1vv	1vv	1vv	1vv
1ww	1ww	1ww	1ww	1ww
1xx	1xx	1xx	1xx	1xx
1yy	1yy	1yy	1yy	1yy
1zz	1zz	1zz	1zz	1zz
1aa	1aa	1aa	1aa	1aa
1bb	1bb	1bb	1bb	1bb
1cc	1cc	1cc	1cc	1cc
1dd	1dd	1dd	1dd	1dd
1ee	1ee	1ee	1ee	1ee
1ff	1ff	1ff	1ff	1ff
1gg	1gg	1gg	1gg	1gg
1hh	1hh	1hh	1hh	1hh
1ii	1ii	1ii	1ii	1ii
1jj	1jj	1jj	1jj	1jj
1kk	1kk	1kk	1kk	1kk
1ll	1ll	1ll	1ll	1ll
1mm	1mm	1mm	1mm	1mm
1nn	1nn	1nn	1nn	1nn
1oo	1oo	1oo	1oo	1oo
1pp	1pp	1pp	1pp	1pp
1qq	1qq	1qq	1qq	1qq
1rr	1rr	1rr	1rr	1rr
1ss	1ss	1ss	1ss	1ss
1tt	1tt	1tt	1tt	1tt
1uu	1uu	1uu	1uu	1uu
1vv	1vv	1vv	1vv	1vv
1ww	1ww	1ww	1ww	1ww
1xx	1xx	1xx	1xx	1xx
1yy	1yy	1yy	1yy	1yy
1zz	1zz	1zz	1zz	1zz

1	water	100	--	--	7.2	<10	110	10,000,000	480,000,000										
2	water	100	--	--	7.3	9,100	23,000	550,000	560,000,000	24,000,000									
3	--	--	Na <sub>2</sub> EDTA	0.8	6.6	5,900	6,900	7,300	88,000	12,000									
4	--	--	citrice acid	0.8	4.2	6,500	4,200	3,500	1,300	640									
5	nisin	0.0001	--	--	7.2	<10	130	9,100,000	45,000,000										
6	nisin	0.0010	--	--	7.3	<10	20	<10	3,700,000	41,000,000									
7	nisin	0.0050	--	--	7.2	<10	<10	<10	9,500	20,000,000									
8	nisin	0.0125	--	--	7.1	<10	<10	<10	220	+									
9	nisin	0.0001	Na <sub>2</sub> EDTA	0.8	6.5	<10	<10	<10	<10	110									
10	nisin	0.0010	Na <sub>2</sub> EDTA	0.8	6.7	<10	<10	<10	<10	110									
11	nisin	0.0050	Na <sub>2</sub> EDTA	0.8	6.7	<10	<10	<10	<10	110									
12	nisin	0.0125	Na <sub>2</sub> EDTA	0.8	6.7	<10	<10	<10	<10	110									
13	nisin	0.0001	citrice acid	0.8	4.4	<10	<10	<10	<10	110									
14	nisin	0.0010	citrice acid	0.8	4.2	<10	<10	<10	<10	110									
15	nisin	0.0050	citrice acid	0.8	4.6	<10	<10	<10	<10	110									
16	nisin	0.0125	citrice acid	0.8	4.3	30	<10	<10	<10	110									

2,800,000.

[0075] Examples 1 and 2 are control examples (not of the invention). In Examples 1 and 2 the test solutions were deionized water, which were mixed with broth as described above (except Example 1 was not inoculated) and run as controls. Example 2 was inoculated and run as controls. The test results indicate that for the uninoculated control (Example 1) there were no significant levels of Listeria present during the 48 hour test period and that growth of total aerobic bacteria was unapparent until the 8 hour test period when such growth proceeded at a rapid pace from the 8

hour period through the 24 hour and 48 hour test periods. The innoculated deionized water control (Example 2) exhibited a lag phase from the initial count of about 31,000 cfu per ml through the 4 hour test sample (37,000 cfu per ml) followed by explosive growth at 8 hours (10,000,000 cfu per ml) and 24 hours (300,000,000 cfu per ml), followed by a die off phase at 48 hours (7,700,000 cfu per ml). This die off phase following explosive growth is believed due to factors related to the immediately preceding high growth such as exhaustion of nutrients or production by the test bacteria (*Listeria*) of inhibiting waste products. Similarly, total aerobic bacteria counts exhibit a period of slow growth followed by explosive growth and then a "die off". In making the total aerobic bacterial count for Examples 1-16, there was evidence of sporeforming bacilli in many examples. Typically, these organisms represent the difference between the total aerobic and *Listeria* bacteria counts.

[0076] Two different chelating agents, namely (1) the disodium salt of EDTA ( $\text{Na}_2\text{EDTA}$ ), and (2) citric acid were tested for antibacterial activity by placing 0.8 weight percent of each in deionized water and innoculating as described above. Example 3 ( $\text{Na}_2\text{EDTA}$ ) was found to inhibit growth of *Listeria* organisms with a maximum number of organisms determined at 24 hours followed by a die off phase at 48 hours. Example 4 (citric acid) was also effective to kill and inhibit *Listeria* although the level of organisms fluctuated over the 48 hour test period with a high average count of 35,000 cfu per ml reported at 8 hours. With respect to total aerobic bacteria counts,  $\text{Na}_2\text{EDTA}$  was inhibitory with a reported high average plate count of 88,000 cfu per ml at 8 hours compared to 560 million cfu per ml for the innoculated control (Example 2). Citric acid was very effective; it produced a steady reduction in the number of total aerobic bacteria over the test period from an initial average count of 6,500 cfu per ml to a low of 640 cfu per ml at 48 hours. The effectiveness of citric acid may have been due at least in part to a pH effect where low pH may restrict bacterial growth as known in the art.

[0077] In Examples 5-16 test solutions utilized various concentrations of nisin alone and with citric acid and  $\text{Na}_2\text{EDTA}$ . The average *Listeria* plate counts for all of these examples were less than 10 cfu per ml for all test periods including the zero hour test conducted immediately following innoculation. Example 2 (the innoculated control) as well as Examples 3 and 4 (containing only chelating agents) were all determined as having average *Listeria* counts of at least 10,000 cfu per ml immediately following innoculation (0hr). Therefore, it would appear that all of the similarly innoculated samples containing nisin under these test conditions acted to kill substantially all *Listeria* upon mixing. That no growth of *Listeria* was seen following the initial test period may indicate either initial eradication or a significant reduction in *Listeria* followed by very effective inhibition.

[0078] The results for the total aerobic bacteria plate counts for Examples 5-16 demonstrate the effect of nisin concentration in the absence and presence of a chelating agent upon total bacteria growth.

[0079] Examples 5-8 were test solutions of varying concentrations of nisin (in the form of Nisaplin brand nisin preparation available from Aplin & Barrett Ltd.) in deionized water. Examples 5-8 all show an initial kill of the innoculated bacteria to a level of less than 10 cfu for each concentration of nisin. The total aerobic bacteria counts obtained by plating the innoculated test solutions onto nonselective tryptic soy agar should be a mixture of the intentionally added *Listeria* and incidental contamination of other microorganisms. By comparison, Examples 2-4 all show initial counts of from 5,900-9,100 cfu per ml, whereas the uninoculated control (Example 1) had an initial count of less than 10 cfu per ml.

[0080] Comparison of Example 5 which contains 1 ppm nisin to the innoculated control (Example 2) shows that nisin is effective, particularly initially to kill aerobic bacteria and control at least initially the growth of aerobic bacteria. However, by the 24 hour test period, the 8 hour average count of 130 cfu per ml for Example 5 had grown explosively to 9,100,000 cfu per ml. This growth is less than that for the innoculated control (Example 2) and about the same as that for the uninoculated control (Example 1) at 24 hours. Rapid growth of total aerobic bacteria continued in Example 5 resulting in an average plate count of 45,000,000 cfu per ml at 48 hours. Comparison of Example 5 with Examples 6-8 demonstrates that increasing the concentration of nisin will act to delay onset of the explosive growth phase for total aerobic bacteria and reduce the average total aerobic bacteria count for each time period relative to the other test solutions containing less nisin.

[0081] Examples 9-12 paralleled Examples 5-8 in nisin concentration, but also contains 0.8 weight percent of a chelating agent, the disodium salt of EDTA (hereinafter  $\text{Na}_2\text{EDTA}$ ). The results for the average total aerobic bacteria plate counts are, except for 110 cfu per ml at 48 hours for Example 9, all less than 10 cfu per ml. Thus a comparison of e.g. the 24 hour average bacteria counts for Example 3 (0.8 wt %  $\text{Na}_2\text{EDTA}$ ), Example 5 (1 ppm nisin), and Example 9 (the combination of 1 ppm nisin and 0.8  $\text{Na}_2\text{EDTA}$ ) show average total aerobic bacteria counts of 88,000 cfu per ml, 9,100,000 cfu per ml and <10 cfu per ml, respectively. The surprising reduction of bacteria to less than 10 cfu per ml for the combination of nisin and chelating agent is unexpected. Nisin and chelating agents such as  $\text{Na}_2\text{EDTA}$  appear to act synergistically to reduce the average number of total aerobic bacteria as shown by comparison of the 24 hour and 48 hour data of Examples 3, and 5-12. In Examples 13-16, a second chelating agent was tried in combination with nisin. These examples were similar to Examples 5-8, but each also contained 0.8 weight percent citric acid. Except for an initial average count of 30 cfu per ml for Example 16, all of these test solutions which contained the combination of citric acid and nisin had average total aerobic plate counts of less than 10 cfu per ml. The above test results dem-

onstrate the antibacterial activity of the individual chelating agents and nisin, as well as the surprisingly and unexpectedly good activity of the combination of nisin and chelating agent against total aerobic bacteria levels. This suggests that the combination of nisin with a chelating agent such as Na<sub>2</sub>EDTA or citric acid works with an unexpected efficiency to kill and inhibit bacteria and may therefore be applied to a foodstuff to dramatically improve shelf life.

- 5 [0082] The above Examples 1-16 demonstrate the effectiveness of various antimicrobial agents against pathogenic and aerobic bacteria. Unexpectedly, the combination of nisin and a chelating agent such as Na<sub>2</sub>EDTA or citric acid was shown to be surprisingly effective against total aerobic bacteria relative to use of either component alone.

#### EXAMPLES 17-31

- 10 [0083] The following examples 17-31 demonstrate the effectiveness of different bactericidal compositions against Listeria when used according to a method which is not of the invention.
- [0084] Various antimicrobial agents applied to foodstuffs such as wiener sausages were tested for effectiveness against subsequent spoilage. Freshly prepared skinless (casing removed) pasteurized frankfurters typically will have surface bacteria of less than 1,000 cfu per frankfurter directly following vacuum packaging during typical commercial manufacturing processes. When bacterial counts reach the 10<sup>7</sup> to 10<sup>8</sup> or higher order of magnitude of cfu per frankfurter, then spoilage is typically visually evident. Common spoilage bacteria for vacuum packaged refrigerated processed meats includes lactobacillus. In particular, the effectiveness of various solutions in protecting foodstuffs dipped therein against growth of pathogenic bacteria such as *Listeria monocytogenes* was tested.
- 15 [0085] Frankfurters formed from typical meat emulsion and processing were utilized. Frankfurters were prepared by stuffing a beef/pork meat emulsion into E-Z Peel NOJAX® Brand cellulose casings (commercially available from Viskase Corporation of Chicago, Illinois) and cooking (approximately 1 hour) in a gas-fired, humidity controlled smoke house at a relative humidity of about 20% until the frankfurters reached an internal temperature of at least 160°F (71°C) under conditions of no added smoke. The casing was then peeled off by a commercial peeler and discarded. The peeled frankfurters were stored in a polyethylene bag at about 4°C briefly until microbiological testing began. The meat emulsion was made from the ingredients listed in Table A by chopping and mixing for about five minutes in a commercial bowl chopper and then mincing through a commercial emulsion mill to achieve a uniform meat emulsion. A chemical analysis of the pasteurized frankfurters revealed 56.9% moisture, 27.2% fat, 12.2% protein, 2.5% ash, 1.90% salt, 65 ppm sodium nitrite and a frankfurter surface pH of 6.40.
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- 25
- 30

Table A

Beef/Pork Emulsion			
Ingredients	Weight		
	lb.	(Kg)	
Beef Chuck	30	(13.5)	
Regular Pork Trimmings	20	(9)	
Water/Ice	12.5	(5.6)	
Salt	1.125	(0.506)	
Dextrose	1.0	(0.45)	
Frankfurter Spices	0.5	(0.225)	
Prague Powder (Sodium nitrite)	0.125	(0.05625)	

- 45 [0086] Refrigerated frankfurters stored at 40°F (4°C) were surface coated with test solutions by submerging individual frankfurters in a test liquid for about 30 seconds with as little handling as possible, followed by a period of about 30 seconds during which each frankfurter was held vertically to drain. The coated frankfurters were then inoculated (except for an uninoculated control) with a mixture of three strains of pathogenic *Listeria monocytogenes* (which were cultured from strains isolated from either a meat product or meat plant) at a level of approximately 10,000 - 30,000 colony forming units (cfu) per frankfurter. Immediately following inoculation, frankfurters from each example were tested by washing with a sterile buffer which was then plated using methods described above into both nonselective tryptone glucose yeast (TGY) agar and *Listeria* selective LPM agar and incubated to determine the presence of total aerobic bacteria and *Listeria*.
- 50 [0087] Following inoculation, frankfurters were individually packaged in commercially available PERFLEX® 51B Barrier Bags (manufactured by Viskase Corporation of Chicago, Illinois). These bags were evacuated and heat sealed under high vacuum with a commercial evacuator/sealer to provide an oxygen and moisture barrier to the environment. The test samples were stored at ambient temperatures (about 25°C) for 2 days and then tested for total bacteria and
- 55

Listeria counts as described above for samples following inoculation. The test solutions and bacteria counts are reported in Table 2.

Table 2

Example No.	Test Solution Components		(cfu/frankfurter)		Listeria		Total Aerobic Bacteria	
	No.	A	Amount B	Amount of B (wt.%)	Initial	Day 2	Initial	Day 2
17	water <sup>+</sup>	100	—	—	<10	<10	2,600	590,000 e
18	water <sup>+</sup>	100	—	—	10,000	38,000,000 e	32,000	65,000,000 e
19	—	—	Na <sub>3</sub> EDTA	3.0	36,000	31,000,000 e	61,000	43,000,000 e
20	nisin*	0.01	—	—	<10	<10	940	51,000,000 e
21	nisin <sup>**</sup>	0.00066	Na <sub>3</sub> EDTA	3.0	<10	1,800,000 e	1,200	53,000,000 e
22	nisin*	0.0034	Na <sub>3</sub> EDTA	3.0	<10	1,800 <sup>+</sup>	6,400	62,000,000 e
23	nisin*	0.01	Na <sub>3</sub> EDTA	3.0	<10	800	1,400	75,000
24	nisin <sup>**</sup>	0.0057	—	—	<10	1,200,000 e	2,700	53,000,000 e
25	nisin <sup>**</sup>	0.00066	Na <sub>3</sub> EDTA	3.0	2,300	2,300,000 e	4,600	79,000,000 e
26	nisin <sup>**</sup>	0.0034	Na <sub>3</sub> EDTA	3.0	60	340,000	1,300	42,000,000 e
27	nisin <sup>**</sup>	0.0057	Na <sub>3</sub> EDTA	3.0	30	26,000	670	79,000
28	—	—	citric acid	3.0	4,800	1,300,000 e	6,500	7,600,000 e
29	nisin*	0.01	cyclodextrin 3.0	21,000	<10	800	60	8,100
30	—	—	cyclodextrin 3.0	21,000	2,800,000 e	37,000	46,000,000 e	
31	nisin*	0.01	cyclodextrin 3.0	<10	27,000	1,900	9,300,000 e	

e - estimate

+ Deionized water

++ Average of two plates only; third plate covered with *Staphylococcus* growth preventing Listeria count.

\* This nisin was added as a preparation produced from fermentation of milk available in powdered form from Apolin & Barrett Ltd. of Trowbridge, England under the trademark "Nisiprin". (1.0 weight percent of the nisin preparation Nisiprin contains 0.025 weight percent of nisin).

\*\* This nisin was also added as a preparation produced in skim milk according to generally known procedures in the art of preparing nisin by culturing of *Streptococcus lactis* in skim milk.

[0088] In Examples 17-31, the antimicrobial agents were dissolved or suspended in deionized water. The test solutions reported in Table 2 were all water based.

[0089] Example 17 differed from the other examples in that its frankfurters were dipped in a deionized water sample only and were not subsequently inoculated with Listeria organisms. Example 17 was run as an uninoculated control (not of the invention) to examine the growth of any background organisms already present e.g. on the frankfurters, or introduced by incidental contamination. The results for Example 17 indicate that there were no significant levels of Listeria detected during the two day test period while the average plate count for total aerobic bacteria increased from

2,600 to an estimated 590,000 cfu per frankfurter.

[0090] Example 18 was run as an inoculated control (not of the invention) with deionized water as the test solution. This Example was identical to Example 17 except that the dipped frankfurters were inoculated with Listeria organisms. Over the two day test period, growth of Listeria was explosive reaching an estimated average plate count of 38,000,000 cfu per frankfurter. The total aerobic bacteria count showed a similar explosive growth.

[0091] In Example 19, a 3 weight percent solution of the trisodium salt of EDTA did not appreciably affect either the Listeria growth or the total aerobic growth on frankfurters over the two day test period.

[0092] In Examples 20-23 various concentrations of nisin were tested alone and in combination with the chelating agent Na<sub>3</sub>EDTA on frankfurters. In these examples nisin was added as a preparation produced from fermentation of milk. This nisin preparation is commercially available under the brand name "Nisaplin" from Aplin & Barrett of Trowbridge, England. In the test solutions to obtain, for example, 0.01 weight percent of nisin it was necessary to add 0.4 weight percent of the nisin preparation (Nisaplin).

[0093] Although all of the test solution coated frankfurters of Examples 18-31 were believed to be initially inoculated with at least about 10,000 cfu per frankfurter of Listeria, the average initial plate counts for Listeria for Examples 20-23 were all less than 10 cfu. These low initial counts are believed to indicate that substantial numbers of Listeria were killed upon contact with the nisin-containing coating. All of the nisin containing coating solutions were effective at reducing growth of Listeria over the two day period with the solutions containing high amounts of nisin being more effective at inhibiting Listeria. Example 20 in which the frankfurter coating contained nisin alone at a test solution level of 100 ppm appeared to be most effective over the two day period. However, this may have been due to either an initial eradication, or a significant reduction followed by very effective inhibition. The test results for total aerobic bacteria suggest that 100 ppm nisin and 3.0 weight percent of Na<sub>3</sub>EDTA act synergistically to hold down total aerobic bacterial growth on coated, cooked or pasteurized meat surfaces as seen by comparison of Example 23 with Examples 19 and 20.

[0094] In Examples 24-27, a noncommercial nisin preparation was utilized. This nisin preparation was made by culturing *Streptococcus faecis* in skim milk using commonly known procedures. Example 24 tested frankfurters coated with a solution of nisin at 52 ppm without the chelating agent Na<sub>3</sub>EDTA. Comparison of results for Example 24 with Examples 19 and 27 indicate that use of the combination of nisin and the chelating agent Na<sub>3</sub>EDTA produced a surprising and unexpected reduction in both average plate counts for Listeria and total aerobic bacteria for the two day test period.

[0095] Other chelating agents were examined with unexpectedly good inhibitory and killing action against total aerobic bacteria for the combination of nisin and either citric acid or cyclodextrin. The nisin/cyclodextrin and nisin citric acid combination also demonstrated very good effectiveness against Listeria growth on food surfaces. The cyclodextrin used in these examples was beta-cyclodextrin which is commercially available from American Maize-Products Company of Hammond, Indiana.

[0096] Examples 17-31 demonstrate that a bactericidal composition comprising nisin and a chelating agent such as Na<sub>3</sub>EDTA, citric acid, or cyclodextrin may be utilized to kill and inhibit pathogenic bacteria and prolong food shelf life. The composition comprising a combination of nisin and chelating agent appears to be useful as a food preservative. Here the solution was applied to frankfurters surfaces by dipping, but it is believed that other methods of application may be employed, as discussed previously, such as spraying, mixing or contact with a releasably coated film and that the inventive combination may be employed not only with processed meat, but other foods including fruits, vegetables, grain products, dairy products, eggs, as well as meats, poultry and fish. The composition is believed to have utility for fresh, raw, cooked, pasteurized and sterilized food products. Synergistic efficiency in killing and inhibiting pathogenic and food spoilage organisms is demonstrated by the above test results.

#### 45 Examples 32-43

[0097] Examples 32-43 demonstrate the effectiveness of different bactericidal compositions against Listeria when used according to a method which is not of the invention.

[0098] Various antimicrobial agents were applied to frankfurters by dipping each into water based test solutions containing the agents. The dipped frankfurters were inoculated with bacteria and tested for surface growth of bacteria over time. The procedures for this test were substantially the same as those followed for Examples 17-31 above except as indicated below. The meat emulsion utilized here was substantially the same recipe used for Examples 17-31 except that no dextrose was used in the meat emulsion for Examples 32-43. The frankfurter cooking/processing conditions were the same except that the relative humidity was 25% and the frankfurters were cooked until reaching an internal temperature of 162°F (72°C). A chemical analysis of the now pasteurized frankfurters revealed a surface pH of 6.36, and 56.3% moisture, 28.7% fat, 12.4% protein, 2.6% ash, 1.94% salt and 56ppm of sodium nitrite. Although no smoke was added, a smoke analysis was done which indicated 24.6 mg of acid, 0.3 mg phenol and 7.1 mg of carbonyl compounds all per 100g of cooked frankfurter. These amounts were believed due to a residual build-up of smoke

constituents in the smokehouse.

[0099] The frankfurters were coated in the test solutions by immersion for thirty seconds followed by draining for thirty seconds. The coated frankfurters were then inoculated with a mixture of three strains of pathogenic Listeria monocytogenes by pipetting 0.05 ml (about 100 cells) of inoculum onto each frankfurter. The inoculum was spread with a sterile cotton swab. The frankfurters were then packaged in two layers of four into commercially available PER-FLEX® 51B Barrier Bags (manufactured by Viskase Corporation of Chicago, Illinois). These bags of thermoplastic film were evacuated and heat sealed under high vacuum with a commercial evacuator/sealer to provide an oxygen and moisture barrier to the environment. Separate sets of packages were prepared for frankfurters coated with each test solution. Each sealed package of eight frankfurters was stored at about 40°F (4.4°C). Triplicate packages were analyzed initially (Day 0) and at 14, 28 and 42 days of storage. For assaying, one frankfurter was aseptically removed from each package being tested and placed in a bag with 10 ml. of phosphate buffer, then shaken to rinse off bacterial cells adhering to the frankfurter surface. Serial decimal dilutions were plated out on LPM agar and TGY agar as for examples 17-31 above. The arithmetic average plate count results from the three replicate packages tested are reported in Table 3.

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Table 3

Example Test-Solution Components No.	Amount A (wt.%)	Amount B (wt.%)	Listeria count (cfu/frankfurter)			Total Aerobic Bacteria (cfu/frankfurter)		
			Time (day) of B	Time (day)	Time (day)	Time (day)	Time (day)	Time (day)
32	uninoculated	100%	--	<10	0	28	42	0
33	control <sup>a</sup>	100%	--	340	140,000	1,400,000,000	ND	120
34	--	--	Na <sub>2</sub> EDTA	0.8	190	2,600,000,000	ND	100
35	propylene	2.0	--	340	340,000	1,200,000,000	ND	180
	glycol							
36	propylene glycol	2.0	Na <sub>2</sub> EDTA	0.8	240	44,000	3,000,000,000	ND
37	propylene glycol	1.8	Charbol-10	0.5	990	300,000 <sup>++</sup>	3,200,000,000	ND
38	sodium benzoate	0.5	Na <sub>2</sub> EDTA	0.8	940	66,000	28,000,000	ND
39	potassium sorbate	0.25	Na <sub>2</sub> EDTA	0.8	260	190,000	1,800,000 <sup>a</sup>	ND
40	lysozyme	0.05%	Na <sub>2</sub> EDTA	0.8	460	10,000	120,000,000	ND
41	parabons**	0.3	Na <sub>2</sub> EDTA	0.8	140	21,000	1,300,000,000	ND
42	nisin	0.01	Na <sub>2</sub> EDTA	0.8	<10	20	<10	<10
43	nisin	0.025	Na <sub>2</sub> EDTA	0.8	<10	<10	<10	<10

ND Not Determined

<sup>a</sup> Butterfield's Buffered Phosphate Diluent.<sup>\*\*</sup> Also included as third component 1.8 wt.% propylene glycol.<sup>+</sup> The three plate counts were <10cfu; 22,000cfu; and 83,000,000cfu. Two of the three plate counts were too numerous to count at the dilution plated; the third plate count is reported.<sup>++</sup> Average of two plates; the third plate was 16,000,000,000cfu.<sup>a</sup> Average of two plates; the third plate was 2,500,000,000cfu.<sup>b</sup> Average of two plates; the third plate count was 3,900cfu.<sup>c</sup> The three plate counts were: <10 cfu; 230 cfu; and 70,000 cfu.<sup>cc</sup> Average of two plates; the third plate count was <10 cfu.

[0100] The frankfurters for Examples 32 and 33 were coated by dipping each frankfurter into a solution of Butterfield's buffered phosphate diluent which contained about 42.5 ppm of potassium orthophosphate in deionized water adjusted to a pH of 7.2. Examples 32 and 33 differed in that only the frankfurters of Example 33 were inoculated with Listeria. Therefore, Example 32 served as an uninoculated control (not of the invention) and Example 33 served as an inoculated control (not of the invention) similar to Examples 17 and 18 above. Butterfield's buffered phosphate diluent was utilized to minimize any disruption due to osmotic forces to any bacteria already present or added. The results indicate no significant levels of Listeria over the 42 day test period for the uninoculated control while the average plate count for total aerobic bacteria climbed to 3,200,000 cfu per frankfurter by the 42 day assay. Frankfurters from the inoculated control (Example 33) showed rapid growth of Listeria from an initial average plate count of 340 cfu per frankfurter to an average of 1,400,000,000 cfu per frankfurter at 28 days. The bacterial plate count for the 42 day sample was not determined due to excessively high numbers of bacteria as determined by visual examination of the packages which revealed cloudiness of the fluid contained within the evacuated package. This cloudiness is known to those skilled in the art of food microbiology to indicate extremely high levels of bacteria. The excessive bacteria numbers at 42 days were apparent in all the examples except for the uninoculated control (Example 32) and Examples 42 and 43 discussed below. Results of the total aerobic mesophilic plate count show that the growth of total aerobic bacteria which included both Listeria (a facultative anaerobe) and any incidental bacterial increase from an average of 120 cfu per frankfurter to an average of 1,900,000,000 cfu per frankfurter at the 28 day assay.

[0101] In Examples 34-41, water based solutions of the disodium salt of EDTA were tested. Na<sub>2</sub>EDTA in solution was tested on frankfurters alone and in combination with propylene glycol, sodium benzoate, potassium sorbate, lysozyme and as a three component system with propylene glycol and parabens. Propylene glycol was also tested alone and with a commercially available liquid smoke sold under the brand name CharSol® C-10 by Red Arrow Products Co. of Manitowoc, Wisconsin. All of the frankfurters coated with these test solutions showed unacceptably high bacterial growth at the end of the 42 day test period. However, Examples 34, 37-40 were of some benefit in inhibiting the growth of bacteria as shown by the reduced average total aerobic bacteria counts through the 28 day assay relative to the uninoculated control, but only the lysozyme, sodium benzoate, and potassium sorbate containing solutions of Examples 40, 38 and 39, respectively, showed any effect at producing logarithmic reductions in average Listeria plate counts at 28 days.

[0102] Examples 42 and 43 tested water based solutions containing 100 ppm and 250 ppm of nisin (the nisin was added in the form of Nisaplin) in combination with 0.8 weight percent of Na<sub>2</sub>EDTA as an antibacterial coating for pasteurized frankfurters. These coatings were effective against the inoculation of frankfurters with pathogenic Listeria, reducing the initial average plate count to less than 10 cfu per frankfurter and maintaining an average plate count of 20 cfu or less per frankfurter for the entire 42 day test period. Use of Listeria selective LPM agar may reduce, through the selective nature of the agar, the number of Listeria organisms originally present. Therefore, the total aerobic bacteria count was performed using a nonselective standard method agar such as TGY agar. Counts made for total aerobic bacteria include not only Listeria colonies but also any incidental colonies from other bacteria which may grow in competition with or in addition to the Listeria such as Staphylococcus. The average plate counts for total aerobic bacteria for Examples 42 and 43 indicate a surprising logarithmic reduction in organisms relative to the uninoculated control of Example 33. Average plate counts were not only 10 or less cfu per frankfurter initially and at 14 days, but the 28 days counts were <10, <10, and 3,900 cfu per frankfurter for Example 42 and <10, 230, and 70,000 cfu per frankfurter for Example 43 compared to 80 million, 440 million and 5.2 billion cfu per frankfurter for the three inoculated control plates (average - 1.9 billion cfu). At 42 days the 100 ppm nisin and Na<sub>2</sub>EDTA solution coated frankfurters had an average plate count of less than 10 cfu per frankfurter, while the three plates assayed for the 250 ppm nisin and Na<sub>2</sub>EDTA solution coated frankfurters of Examples 43 were counted at <10, 270,000; and 1,300,000 cfu per frankfurter. Thus, the 42 day total aerobic bacteria counts for the inoculated frankfurters of Example 42 and 43 may be favorably compared with the three plate counts of 130,000; 180,000; and 9,200,000 cfu per frankfurter (average - 3.2 million cfu) assayed at 42 days for the uninoculated control of Example 32. These remarkable results further indicate that compositions containing nisin and a chelating agent may be used to protect against growth of pathogenic and food spoilage bacteria over long periods of time at reduced temperatures. Thus, food preservation may be enhanced with longer times of preservation. The compositions may be employed as a dip solution or may be sprayed, mixed with the foodstuff or may be coated onto packaging films for subsequent food surface contact.

#### Examples 44-53

[0103] Examples 44-53 assessed the antimicrobial potential of using coated frankfurter casings for control of Listeria monocytogenes and other naturally occurring microorganisms on frankfurters which have had the casings removed therefrom prior to vacuum packaging.

[0104] Frankfurters were made utilizing the beef/pork emulsion recipe and procedure described above for Examples 17-31 except that the dextrose level in the recipe for Examples 44-53 was one half that reported in Table A. Processing

was similar, but at 25% relative humidity and the frankfurters were cooked until reaching an internal temperature of about 160°F (71°C). A further exception to the above procedure for making frankfurters was that the casing used was internally coated with the test components by addition of nisin and/or the di- or tri-sodium salt of EDTA to a stirring solution prior to stirring of the casing. The test components were added in the amounts sufficient to give the weight percentages listed in Table 4 which are based upon the total weight of the finished stirred remoisturized casing. Examples 44 and 45 were run, respectively, as uninoculated and innoculated controls (not of the invention) and utilized commercially available casing containing substantially the same stirring solution components as Examples 46-53 except without either nisin or an EDTA sodium salt. Typical stirring solutions are disclosed e.g. in U.S. Patent No. 3,898,348 which is hereby incorporated by reference. Following removal of the casings, the frankfurters were refrigerated at about 4°C until beginning microbiological testing. The freshly made refrigerated frankfurters were inoculated with a mixture of Listeria monocytogenes and assayed initially (Day 0), and at day 7, 14, 28 and 42 following procedures described above for Examples 32-43 except as noted herein. The inoculation of frankfurters with Listeria was made at a level of at least about 1000 organisms per frankfurter. The inoculated frankfurters were stored eight to a package with separate sets of packages for frankfurters of each test casing similar to the procedures for Examples 32-43 and stored at about 40°F (4.4°C) until assayed. The arithmetic average of the assay results are reported in Tables 4a and 4b.

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Table 4a

Example No.	Test Components	Amount of A (wt.%)	B	Amount of B (wt.%)	Listeria count (cfu/frankfurter)					
					Time (day)	0	7	14	28	<10
44	uninoculated	—	—	—	<10	120,000	6,300,000	25,000,000**	32,000	1,500,000
	control	—	—	—	—	8,500	7,300	620	32,000	740,000
45	innoculated	—	—	—	—	—	—	—	—	200,000,000
46	control	—	—	—	—	—	—	—	—	—
47	nisin	0.0135	Na <sub>3</sub> EDTA	0.8	8,500	6,800	620	32,000	32,000,000**	300,000,000
48	nisin	0.0135	Na <sub>3</sub> EDTA	0.8	4,700	38,000	28,000	32,000,000	32,000,000	300,000,000
49	nisin	0.0060	Na <sub>3</sub> EDTA	0.8	11,000	3,100	3,600	66,000	66,000	20,000,000+
50	nisin	0.0026	Na <sub>2</sub> EDTA	0.8	5,000	8,300	37,000	22,000++	22,000++	4,900,000
51	nisin	0.0005	Na <sub>3</sub> EDTA	0.8	6,100	3,100	4,700*	aa	aa	8,500,000
52	nisin	0.0005	Na <sub>3</sub> EDTA	0.8	5,900	29,000	120,000	51,000,000	51,000,000	300,000,000
53	nisin*	0.0005	Na <sub>3</sub> EDTA	0.8	5,500	5,500	310,000b	23,000	23,000	21,000bb

\* The nisin in Examples 47-52 was Nisaplin brand nisin preparation; Example 53 used a nisin preparation made by culturing *streptococcus lactic* in skim milk.

\*\* Average of two plates; third plate count was 35,000 cfu.

+ Average of two plates; third plate count was 44 billion cfu.

++ Average of two plates; the third plate count was >1,000,000 cfu.

a Average of two plates; the third plate count was 2,400,000 cfu.

aa The three plate counts were 130,000; >10,000,000; and 350,000,000 cfu.

b Average of two plates; the third plate count was 470 cfu.

bb Average of two plates; the third plate count was 3,300,000 cfu.

Table 4b

Example No.	Test Solution Components		Amount of B (wt.%)	Time (day)	Total Aerobic Bacteria Count (cfu/frankfurter)		
	A	B			0	7	14
44	uninoculated	--	--	900	2,000	360	4,300,000
	control	--	--				22,000
45	innoculated	--	--	6,700	6,100	210,000	3,600,000
	control	--	--				680,000,000
46	--	Na <sub>3</sub> EDTA	0.8	5,000	2,100	1,700	14,000,000
	nisin	0.0135	--	4,500	3,300	22,000	***
47	nisin	0.0135	Na <sub>3</sub> EDTA	0.8	3,300	1,300	2,800,000
48	nisin	0.0060	Na <sub>3</sub> EDTA	0.8	5,000	1,800	18,000
49	nisin	0.0060	Na <sub>3</sub> EDTA	0.8	3,900	3,100	12,000++
50	nisin	0.0026	Na <sub>3</sub> EDTA	0.8	4,500	3,800	210,000,000
51	nisin	0.0005	Na <sub>3</sub> EDTA	0.8	4,500	24,000	130,000,000
52	nisin	0.0005	Na <sub>3</sub> EDTA	0.8	4,500	42,000	8,200,000
53	nisin*	0.0005	Na <sub>3</sub> EDTA	0.8	4,000 <sup>b</sup>	2,000	120,000
							7,100
							1,200,000,000

\* The nisin in Examples 47-52 was Nisagelin brand nisin preparation; Example 53 used a nisin preparation made by culturing streptococcus lactis in skim milk.

\*\* The three plate counts were 9,700; 2,100,000; and >1,000,000cfu.

+ Average of two plates; the third plate count was 18 billion cfu.

++ Average of two plates; the third plate count was >1,000,000 cfu.

a Average of two plates; the third plate count was 400,000 cfu.

b Average of two plates; there was a laboratory error analyzing the third plate.

[0105] Referring to Tables 4a and 4b, the uninoculated, untreated frankfurters of control Example 44 showed no evidence of Listeria throughout the 42 day test and the average total aerobic plate counts grew from 900 cfu per frankfurter initially to 4,300,000 cfu per frankfurter at day 28, and 22,000 cfu per frankfurter at day 42.

[0106] The innoculated control exhibited an initial average level of Listeria of 8,500 cfu per frankfurter which grew to 200,000,000 cfu per frankfurter by day 42 and had an initial average total aerobic bacteria plate count of 6,700 cfu per frankfurter which grew to 680 million cfu per frankfurter by day 42. Both controls were made using unmodified commercially available E-Z Peel Nojax® cellulose nonfibrous casing manufactured by Viskase Corporation of Chicago, Illinois. Examination of the data for Examples 46-53 indicate that antimicrobial agents applied to casing prior to stuffing with meat emulsion and cooking were transferred to cooked frankfurter surfaces in amounts sufficient to inhibit growth of both Listeria and total aerobic bacteria relative to the innoculated control (Example 45) following removal of the casing from the frankfurter.

[0107] Regular cellulose casings may also be used in the present invention. Such casings may forego chemical peeling aids. Advantageously, such casings may be acid treated, or encased foodstuffs, particularly protein and fat containing foodstuffs such as freshly stuffed sausages, may be showered with an acidic solution prior to heat treatment (cooking or pasteurization). Such acid treatment may have a beneficial effect which enhances or maintains the ability of antimicrobial agents such as nisin either alone or in conjunction with a chelating agent, to protect the encased foodstuff before and after heat treatment and/or casing removal.

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#### Examples I-IV

[0108] Examples I-III are comparative examples not of the invention. Example IV is of the present invention.

[0109] In all of the following examples the food packaging film referred to comprises a nonfibrous, small diameter, regenerated cellulose casing of the type well known in the art for the manufacture of skinless frankfurters, wieners and the like. Such casing may be coated or impregnated with various additives to enhance shelf-life, peelability, etc.

[0110] Dry stock, nonfibrous casing is conventionally shirred into sticks of casing for use on a conventional stuffing machine. During the shirring operation, just prior to the gathering of the casing into pleats, a sprayed solution is uniformly applied at a constant rate to the internal surface of the casing. This is a conventional method (See e.g. U.S. Patent 3,462,794 which is hereby incorporated by reference) of applying an internal coating to a casing.

[0111] Four different solutions as described in Table I may be applied by the above procedure to provide four different casing samples with each sample having an equivalent controlled amount of solution applied uniformly thereto.

[0112] The solution composition of comparative Example I is a typical solution sprayed on the interior of a casing just prior to shirring. The water moisturizes the casing and also acts as a carrier for the other components which may include a plasticizer and/or humectant such as propylene glycol, a lubricant such as mineral oil, an emulsifier such as a mixture of ethoxylated monoglycerides sold under the brand name Mazol 80 by Mazer Chemicals, Inc. of Gurnee, Illinois, and a peeling aid such as carboxymethylcellulose.

[0113] The solution of comparative Example II is of the same composition as Example I except that a chelating agent viz the disodium salt of ethylenediaminetetraacetic acid ( $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$ ) is added.

[0114] The solution of comparative Example III is similar to that of comparative Example II except that 0.025 weight percent of the solution comprises an antimicrobial agent in the form of lysozyme.

[0115] The solution of Example IV of the invention is similar to that of comparative Example II except that 1.0 weight percent of Nisaplin is added. Nisaplin is a trademarked commercial nisin preparation made from a pure culture fermentation of nonpathogenic strains of *Streptococcus lactis* belonging to Lancefield Group N with penicillin-free, heat-treated sterilized nonfat milk digest. The fermentation product is concentrated by a foaming process and extracted by salt precipitation under acid conditions and dried by a spray process to produce a mixture which has an activity which is 1/40th of pure nisin. Nisin preparation is further described in the Federal Register Vol. 53, No. 66, pp. 11247-11251 (April 6, 1988) which is hereby incorporated by reference. Since 1.0 weight percent of Nisaplin is used, the solution of Example IV contains 0.025 weight percent of nisin. The activity of pure nisin is about  $40 \times 10^6$  IU per gram.

[0116] Each of the above four solutions are uniformly applied to similar but separate samples of casings. Each solution is sprayed at a constant rate which is adjusted for each solution to provide a sprayed casing that has a water moisture content of about 19.3 percent by weight based on the total weight of the moisture coated casing.

[0117] The four samples of shirred casing, each internally coated with a different solution, may be conventionally stuffed with a typical all beef frankfurter meat emulsion on a commercially available machine such as a Frank-A-Matic brand frankfurter stuffing machine manufactured by Townsend Engineering Co. of Des Moines, Iowa to a stuffed diameter of about 21-22 millimeters. The encased wieners may then be collected on racks and placed in a smokehouse for conventional heat treatment.

[0118] A typical heat treatment process includes admitting humidified hot air from a gas fired heater until the smokehouse temperature is increased (usually taking 15-30 minutes) to a dry bulb temperature of about 140°F (60°C) and a relative humidity (RH) of about 25%. Smokehouse dampers are then closed; smoke is admitted and the temperature and humidity held constant at 60°C/25%RH for fifteen minutes. Then admittance of smoke is halted, the smokehouse dampers are allowed to open as needed and the smokehouse temperature is raised (over a time period of usually 15-20 minutes) to a dry bulb temperature of about 160°F (71°C) at a constant relative humidity of about 25% and held

there for fifteen minutes. Then the dry bulb temperature is again raised (usually taking 15-20 minutes) to about 180°F (82°C) at a constant 25% relative humidity and held there for about 45 minutes until the internal temperature of the encased frankfurters reaches 155-160°F (68-71°C).

[0119] After reaching an internal temperature of 155-160°F (68-71°C), the heat is turned off and cold tap water is showered over the frankfurters for about ten minutes whereupon the frankfurters are moved to a brine shower tunnel where the encased frankfurters are showered in a brine (8% salt) water solution at about 25°F (-4°C) for approximately ten minutes until the internal temperature of the frankfurter is cooled to about 35°F (2°C).

[0120] The casing may then be removed from the chilled encased frankfurters by conventional equipment such as an Ranger Apollo peeler to produce "skinless" wieners.

[0121] Skinless frankfurters of each example may now be inoculated with a buffered solution containing three strains of pathogenic Listeria monocytogenes. Listeria may be applied to each frankfurter surface using a sterile swab, which is dipped into the Listeria containing solution and swabbed once across the frankfurter length so that at least approximately 100 and preferably at least 1000 cells of Listeria monocytogenes are deposited on the surface of each frankfurter.

[0122] The inoculated wieners may then be packaged in two layers with each layer having four wieners so that eight wieners are in a multilayer thermoplastic barrier bag having a core oxygen and moisture barrier layer comprising a mixture of vinylidene chloride-methyl acrylate copolymer and vinylidene chloride-vinyl chloride copolymer and outer layers of ethylene-vinyl acetate copolymer on either side of the core. For each example, a plurality of bags each containing eight wieners is evacuated to 29 inches of mercury and heat sealed.

[0123] The packaged inoculated wieners may then be refrigerated at about 40°F (4°C) with samples from packages of each example being tested initially and every two weeks thereafter for six weeks.

[0124] At the above intervals, sample frankfurters may be tested by removing a wiener and washing off any micro-organisms thereon with a sterile buffer. The wash buffer is then tested for (1) a standard plate count with tryptone glucose yeast agar by conventional means and (2) a selective Listeria monocytogenes count using LPM agar and the FSIS method described above for Examples 1-6.

[0125] The initial sampling of frankfurter is expected to confirm the presence of Listeria on all samples and of lysozyme and nisin on the frankfurters from Examples III and IV respectively.

[0126] After both four and six weeks the above comparative Examples I and II are expected to both show growth of live colonies of Listeria monocytogenes relative to the initial inoculation. The lysozyme treated frankfurters of Example III are expected to show fewer viable Listeria organisms compared with the inoculated control samples. The nisin treated frankfurters of Example IV are also expected to show a decrease in viable Listeria organisms relative to the inoculated control wieners at four and six weeks.

[0127] The above comparative Examples I-III and Example IV of the present invention demonstrate the method and film of the present invention.

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## Claims

1. A food packaging film comprising a polymeric film containing a bactericidal composition having an enhanced antimicrobial effect and comprising a combination of a *Streptococcus*-derived bacteriocin or synthetic equivalent antibacterial agent and a chelating agent in an amount effective to kill, prevent or inhibit growth of pathogenic *Listeria monocytogenes* upon contact.
2. A film according to claim 1, wherein said bactericidal composition is impregnated in said film.
3. A film according to claim 1, wherein said bactericidal composition is coated on at least one surface of said film.
4. A film according to one of claims 1 to 3, wherein said film comprises a tubular casing.
5. A film according to one of claims 1 to 3, wherein said film comprises a heat shrinkable bag.
6. A film according to one of claims 1 to 3, wherein said film comprises a non-fibrous, cellulosic, tubular food casing.
7. A film according to one of claims 1 to 6, wherein said film comprises regenerated cellulose.
8. A film according to one of claims 1 to 7, wherein said film is adapted for transferring said bactericidal composition to a food surface in contact therewith in an amount effective to kill, prevent or inhibit growth of pathogenic *Listeria monocytogenes* on said food surface subsequent to removal of said film.

9. A film according to one of claims 1 to 8, wherein said bacteriocin or said antibacterial agent is heat resistant.
10. A film according to one of claims 1 to 9, wherein said bacteriocin is nisin.
- 5 11. A film according to one of claims 1 to 10, wherein said composition is a solution containing said antimicrobial agent as a solute or dispersion.
12. A film according to one of claims 1 to 11, wherein said chelating agent comprises EDTA or its salts.
- 10 13. A film according to one of claims 1 to 12, further comprising additives such as binding agents, buffers, emulsifiers and/or transfer aids.
14. A method of treating a foodstuff surface with a bactericidal composition having an enhanced antimicrobial effect and comprising a combination of a Streptococcus-derived bacteriocin or synthetic equivalent antibacterial agent and a chelating agent in an amount effective to kill, prevent or inhibit growth of pathogenic Listeria monocytogenes upon contact, said method comprising:
  - a) contacting a surface of a foodstuff with film according to claims 1 to 13 containing said bactericidal composition;
  - 20 b) transferring a controlled amount of said bactericidal composition from said film to said foodstuff; and
  - c) removing said film whereby an effective amount of said transferred composition remains on said foodstuff surface to kill, prevent or inhibit growth of pathogenic Listeria monocytogenes thereon.
- 25 15. A method of treating a foodstuff surface with a bactericidal composition having an enhanced antimicrobial effect and comprising a combination of a Streptococcus-derived bacteriocin or synthetic equivalent antibacterial agent and a chelating agent in an amount effective to kill, prevent or inhibit growth of pathogenic Listeria monocytogenes upon contact, said method comprising contacting a foodstuff with a film according to claims 1 to 13 coated with said composition.
- 30 16. A method according to claim 15, wherein a surface of a foodstuff is at least partially coated with said composition.

#### Patentansprüche

- 35 1. Folie (Film) zur Verpackung von Nahrungsmitteln, umfassend einen Polymerfilm mit einer antibakteriellen Zusammensetzung, die eine gesteigerte antimikrobielle Wirkung besitzt und eine Kombination eines Streptococcus-abgeleiteten Bacteriocins oder eines äquivalenten, synthetischen antibakteriellen Mittels und eines Chelatbildners in einer ausreichenden Menge enthält, um bei Kontakt pathogene Listeria monocytogenes abzutöten oder deren Wachstum zu verhindern oder zu inhibieren.
- 40 2. Folie nach Anspruch 1, wobei die antibakterielle Zusammensetzung in der Folie imprägniert ist.
3. Folie nach Anspruch 1, wobei die antibakterielle Zusammensetzung auf mindestens eine Oberfläche der Folie aufgetragen ist.
- 45 4. Folie nach einem der Ansprüche 1 bis 3, wobei die Folie eine schlauchförmige Hülle umfaßt.
5. Folie nach einem der Ansprüche 1 bis 3, wobei die Folie einen wärmeschrumpfbaren Beutel umfaßt.
- 50 6. Folie nach einem der Ansprüche 1 bis 3, wobei die Folie eine faserfreie, schlauchförmige Nahrungsmittelhülle aus Cellulose umfaßt.
7. Folie nach einem der Ansprüche 1 bis 6, wobei die Folie regenerierte Zellulose enthält.
- 55 8. Folie nach einem der Ansprüche 1 bis 7, wobei die Folie geeignet ist, um die antibakterielle Zusammensetzung auf eine Nahrungsmitteloberfläche, die in Kontakt hierzu steht, in einer ausreichenden Menge zu übertragen, um pathogene Listeria monocytogenes auf der Nahrungsmitteloberfläche nach Entfernen der Folie abzutöten oder deren Wachstum zu verhindern oder zu inhibieren.

9. Folie nach einem der Ansprüche 1 bis 8, wobei das Bacteriocin oder das antibakterielle Mittel hitzebeständig ist.
10. Folie nach einem der Ansprüche 1 bis 9, wobei das Bacteriocin Nisin ist.
- 5 11. Folie nach einem der Ansprüche 1 bis 10, wobei die Zusammensetzung eine Lösung ist, die das antimikrobielle Mittel als gelösten Stoff oder in Dispersion enthält.
- 10 12. Folie nach einem der Ansprüche 1 bis 11, wobei der Chelatbildner EDTA oder seine Salze umfaßt.
- 15 13. Folie nach einem der Ansprüche 1 bis 12, außerdem enthaltend Additive wie Bindemittel, Puffer, Emulgatoren und/oder Übertragungsmittel.
14. Verfahren zur Behandlung einer Nahrungsmitteloberfläche mit einer antibakteriellen Zusammensetzung mit gesteigerter antimikrobieller Wirkung, die eine Kombination eines Streptococcus-abgeleiteten Bacteriocins oder eines äquivalenten, synthetischen antibakteriellen Mittels und eines Chelatbildners in einer ausreichenden Menge enthält, um Listeria monocytogenes bei Kontakt abzutöten oder deren Wachstum zu verhindern oder zu inhibieren, wobei das Verfahren umfaßt:
  - 20 a) Inkontaktbringen einer Nahrungsmitteloberfläche mit der Folie nach den Ansprüchen 1 bis 13, die die antibakterielle Zusammensetzung enthält;
  - b) Übertragung einer kontrollierten Menge der antibakteriellen Zusammensetzung von der Folie auf das Nahrungsmittel; und
  - c) Entfernen der Folie, wobei eine wirksame Menge der übertragenen Zusammensetzung auf der Lebensmitteloberfläche verbleibt, um pathogene Listeria monocytogenes dort abzutöten oder deren Wachstum zu verhindern oder zu inhibieren.
- 30 15. Verfahren zur Behandlung einer Nahrungsmitteloberfläche mit einer antibakteriellen Zusammensetzung mit gesteigerter antimikrobieller Wirkung, die eine Kombination eines Streptococcus-abgeleiteten Bacteriocins oder eines äquivalenten, synthetischen antibakteriellen Mittels und eines Chelatbildners in einer ausreichenden Menge enthält, um bei Kontakt Listeria monocytogenes abzutöten oder deren Wachstum zu verhindern oder zu inhibieren, wobei dieses Verfahren beinhaltet, daß das Nahrungsmittel mit einem Film nach den Ansprüchen 1 bis 13, der mit der Zusammensetzung beschichtet ist, in Kontakt gebracht wird.
- 35 16. Verfahren nach Anspruch 15, wobei die Nahrungsmitteloberfläche zumindest teilweise mit der Zusammensetzung beschichtet wird.

#### Revendications

- 40 1. Film d'emballage alimentaire comprenant un film polymère contenant une composition bactéricide ayant un effet anti-microbien accru et comprenant une association d'une bactériocine dérivée de Streptococcus ou d'un agent anti-bactérien équivalent synthétique et d'un agent chélatant en une quantité efficace pour tuer, empêcher ou inhibier la croissance de Listeria monocytogenes pathogènes par contact.
- 45 2. Film suivant la revendication 1, dans lequel la composition bactéricide imprègne ledit film.
3. Film suivant la revendication 1, dans lequel la composition bactéricide est appliquée sous forme d'un revêtement sur au moins une surface dudit film.
- 50 4. Film suivant l'une des revendications 1 à 3, qui comprend une enveloppe tubulaire.
5. Film suivant l'une des revendications 1 à 3, qui comprend un sac thermorétractable.
- 55 6. Film suivant l'une des revendications 1 à 3, qui comprend une enveloppe alimentaire tubulaire, cellulosique non fibreuse.
7. Film suivant l'une des revendications 1 à 6, qui comprend de la cellulose régénérée.

8. Film suivant l'une des revendications 1 à 7, qui est apte au transfert de la composition bactéricide à une surface alimentaire en contact avec ce film en une quantité efficace pour tuer, empêcher ou inhiber la croissance de *Listeria monocytogenes* pathogènes sur ladite surface alimentaire après l'enlèvement dudit film.
- 5 9. Film suivant l'une des revendications 1 à 8, dans lequel la bactériocine ou l'agent anti-microbien est thermorésistant.
10. Film suivant l'une des revendications 1 à 9, dans lequel la bactériocine est la nisine.
11. Film suivant l'une des revendications 1 à 10, dans lequel la composition est une solution contenant l'agent anti-microbien sous forme d'un soluté ou d'une dispersion.
12. Film suivant l'une des revendications 1 à 11, dans lequel l'agent chélatant comprend le EDTA ou ses sels.
13. Film suivant l'une des revendications 1 à 12, comprenant en outre des additifs tels que des liants, des tampons, des émulsifionnats et/ou des adjuvants de transfert.
14. Procédé pour le traitement d'une surface d'une denrée alimentaire avec une composition bactéricide ayant un effet anti-microbien accru et comprenant une association d'une bactériocine dérivée de *Streptococcus* ou d'un agent anti-bactérien équivalent synthétique et d'un agent chélatant en une quantité efficace pour tuer, empêcher ou inhibiter la croissance de *Listeria monocytogenes* pathogènes par contact, ledit procédé comprenant les étapes consistant :
- 25 a) à mettre en contact une surface d'une denrée alimentaire avec un film suivant les revendications 1 à 13 contenant ladite composition bactéricide ;  
b) à transférer une quantité ajustée de ladite composition bactéricide dudit film à ladite denrée alimentaire ; et  
c) à enlever ledit film, ce qui fait qu'une quantité efficace de ladite composition transférée reste sur la surface de ladite denrée alimentaire pour tuer, empêcher ou inhibiter la croissance de *Listeria monocytogenes* pathogènes sur cette surface.
- 30 15. Procédé pour le traitement d'une surface d'une denrée alimentaire avec une composition bactéricide ayant un effet anti-microbien accru et comprenant une association d'une bactériocine dérivée de *Streptococcus* ou d'un agent anti-bactérien équivalent synthétique et d'un agent chélatant en une quantité efficace pour tuer, empêcher ou inhibiter la croissance de *Listeria monocytogenes* pathogènes par contact, ledit procédé comprenant la mise en contact d'une denrée alimentaire avec un film suivant les revendications 1 à 13 revêtu de ladite composition.
- 35 16. Procédé suivant la revendication 15, dans lequel une surface d'une denrée alimentaire est revêtue au moins partiellement avec la composition.

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